Otolith shape differences between *Micropogonias manni* and *M. altipinnis* (Sciaenidae)

by

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Abstract. – Sagitta morphology is much used in sciaenid systematics, as well as in archaeological, paleontological and ecological studies. Although morphometric characteristics of sagitta (length, height, thickness, weight,…) are useful to discriminate some marine and freshwater species, they are not fully conclusive in the case of *Micropogonias altipinnis* and *M. manni*. In order to give new elements for characterizing and discriminating these species we propose here to apply the geometric morphometry technique to sagittae of these species by combining landmarks and sliding semi-landmarks. We measured 40 sagittae of *Micropogonias altipinnis* and 46 sagittae of *M. manni*, and quantified their differences in shape and centroid size. We also tested for an effect of the length and weight of *Micropogonias* specimens on the shape of the sagitta. Our results show that it is possible to discriminate the two species. Moreover, sagitta shape differs significantly between species, while centroid size does not. These results validate the application of our protocol for characterizing and discriminating the shape of the sagitta, and show that the combined use of landmarks and sliding semi-landmarks is a very powerful option to capture the elusive morphological changes present in *Micropogonias* sagittae.

Résumé. – Différences de forme entre les otolithes de *Micropogonias manni* et *M. altipinnis* (Sciaenidae).

La morphologie des sagittae est très utilisée dans la systématique des Sciaenidae, ainsi que dans les études archéologiques, paléontologiques et écologiques. La longueur, la hauteur, l'épaisseur et le poids sont des variables utilisées pour distinguer des espèces marines ou d'eau douce, mais elles ne sont pas discriminantes dans le cas de *Micropogonias altipinnis* et *M. manni*. Nous proposons d'appliquer la morphométrie géométrique aux sagittae de ces deux espèces afin d'apporter de nouveaux éléments pour les caractériser et les discriminer. Nous avons mesuré 40 sagittae de *Micropogonias altipinnis* et 46 sagittae de *M. manni* et avons quantifié leurs différences de conformation et de taille centroïde. Nous avons aussi testé l'effet de la longueur et du poids des spécimens de *Micropogonias* sur la conformation de la sagitta. Nos résultats montrent qu'il est possible de distinguer les deux espèces. De plus, la forme de la sagitta est différente mais pas la taille centroïde. Ces résultats valident l'application de notre protocole pour caractériser et discriminer la forme de la sagitta et indiquent que l'utilisation simultanée de repères et de "sliding semi-landmarks" est une bonne alternative aux mesures traditionnelles pour mettre en évidence les changements morphologiques imperceptibles des sagittae de *Micropogonias*.

Key words
Sciaenidae
Micropogonias
Geometric morphometrics
Sliding semi-landmarks
Species identification
Otoliths

Sciaenids, commonly called drums or croakers, inhabit predominantly marine and brackish environments in shallow waters over sandy and muddy bottoms of the Atlantic, Indian and Pacific Oceans (Chao, 1995; Nelson, 2006). This family includes about 67 genera and 292 species (Eschmeyer and Fong, 2015). The genus *Micropogonias* Bonaparte, 1831 is strictly neotropical (Chao, 1986) and four species are present in the Pacific coast (Chao, 1995): *Micropogonias altipinnis* (Günther, 1864), *Micropogonias ectenes* (Jordan & Gilbert, 1882), *Micropogonias manni* Moreno, 1970 and *Micropogonias megalops* (Gilbert, 1890). This paper focuses on *M. altipinnis* and *M. manni* because they both live in South America and might have had overlapping distribution range in the past. Although they inhabit two different environments in western South America (Fig. 1), *M. altipinnis* is supposed to have been present ca. 10,000 years ago in Northern Chile (Llagostera, 1979), where *M. manni* is reported today. Nowadays, *Micropogonias altipinnis* inhabits the coastal marine habitats from the Gulf of California to the north of Peru (Chao, 1995) and *Micropogonias manni* inhabits brackish to freshwater environments of central Chile (Moreno, 1970). These two species can be distinguished by anatomical traits of their external morphology but have very similar saccular otoliths or sagittae (Fig. 2), which hampers their correct discrimination in paleontology and zooarchaeology.

The sagittae are one of the three different otoliths in the inner ear of teleosts, which participate in the hearing and balance functions in the water environment (Motta *et al.*,

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2009). In most cases, they are basically formed by aragonite crystals arranged in a protein matrix (Lombarte *et al.*, 2006). Because of their species-specific shape (Campana and Casselman, 1993), sagittae can be used as a tool in sciaenid taxonomy and phylogeny (Chao, 1978; Sasaki, 1989; Schwarzhans, 1993; Béarez and Schwarzhans, 2013), and for the identification of species in archaeological or fossil samples (Llagostera, 1979; Scartascini *et al.*, 2009; Béarez *et al.*, 2011), or in the stomach content samples for dietary item identification (Derby and Lovvorn, 1997; Browne *et al.*, 2002).

Although sagitta morphometric measurements (length, height, thickness, or weight) aid to discriminate some marine and freshwater species (Schwarzhans, 1993; Tuset *et al.*, 2003; Cañás *et al.*, 2012; Wakefield *et al.*, 2014), they are not fully conclusive in the case of *Micropogonias altipinnis* and *M. manni* (PB, unpubl. data). The geometric morphometry (GM) method has been successfully applied in many fields of biological research including taxonomy, ecology, evolution and ontogeny (Morais *et al.*, 2013). This method preserves the main geometric properties of specimens, while generating a visual representation and determining shape variables that can be analysed statistically (Adams *et al.*, 2004). Because of its large size in sciaenids, and the rich biological information present on its mesial surface, where the *sulcus acusticus* and the *macula* are in tight contact, the sagitta is a well-suited object for application of GM to quantify the morphological information present in each specimen. GM permits to work quantitatively and qualitatively on biological form more accurately than classical linear measurements (Adams, 2004; Zelditch, 2012). Landmarks are anatomical point locations (Bookstein, 1991) while sliding semi-landmarks are reference points placed along the curves or surfaces (Gunz and Mitteroecker, 2013). These techniques have been successfully applied to distinguish between sciaenid species (Monteiro *et al.*, 2004, 2005) and also to test the relationship between otolith morphology and body size (Waessle *et al.*, 2003; Monteiro *et al.*, 2005).

In the coastal archeological sites of the neotropics, it is frequently difficult to attribute *Micropogonias* sagittae with certainty to either one or other species (Llagostera *et al.*, 1997). In order to provide a reliable tool for archeological material identification along the South American Pacific coast we applied the GM technique to modern *Micropogonias* sagittae.

The aim of this study was to determine the contribution of GM method to the discrimination of modern species. Is

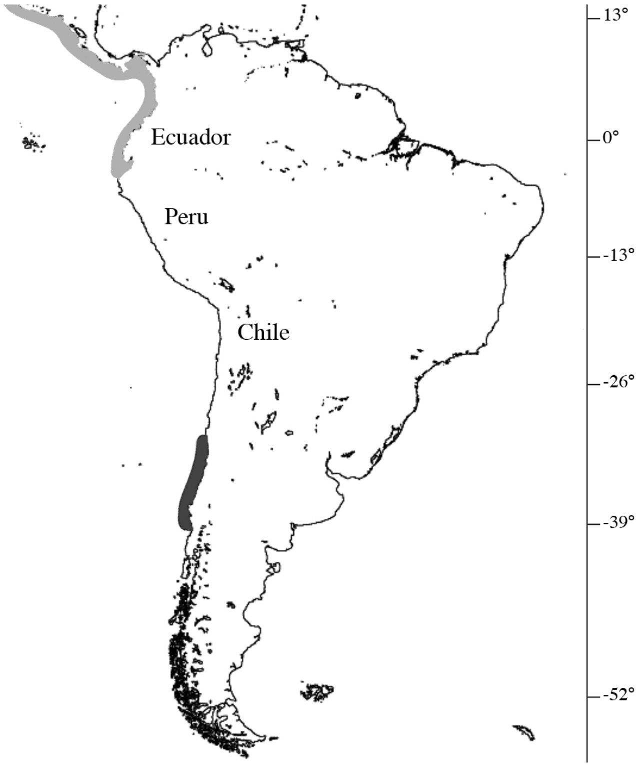

Figure 1. - Current distribution range of *Micropogonias altipinnis* (light grey) and *Micropogonias manni* (dark gray).

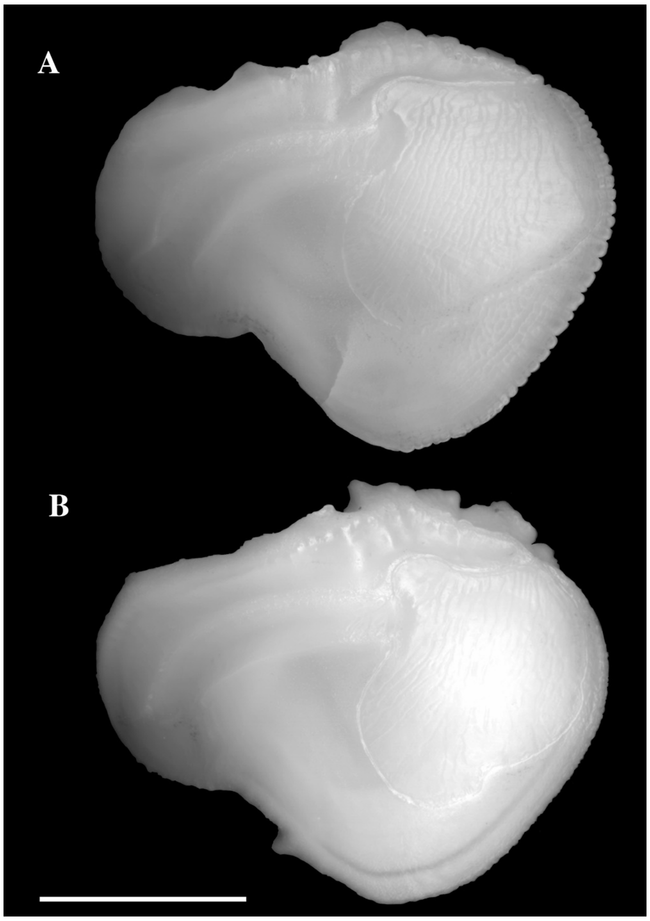

Figure 2. - Left sagitta of *Micropogonias altipinnis* (**A**) and *M. manni* (**B**); mesial view. Scale bar: 5 mm.

it possible to separate modern *Micropogonias* species using sagitta shape? Does *Micropogonias* sagitta shape vary in relation to sex and also with length of specimens?

MATERIALS AND METHODS

We used 40 sagittae of *Micropogonias altipinnis* and 46 of *M. manni* specimens collected from Ecuador and Peru, and from Chile, respectively (Tab. I; Fig. 1). Although standard length (SL) and total length (TL) are generally highly correlated, both measures are commonly used in the literature and were taken into account. Lengths were obtained by measurements with a caliper to the nearest mm; body weight (BW) by using a weighing balance (g) and the sex by dissection. Sagittae were extracted using the guillotine method (Stevenson and Campana, 1992), cleaned from adhering tissues, dried and then preserved in the collection with an inventory number. We followed the morphological terminology of Schwarzhans (1993).

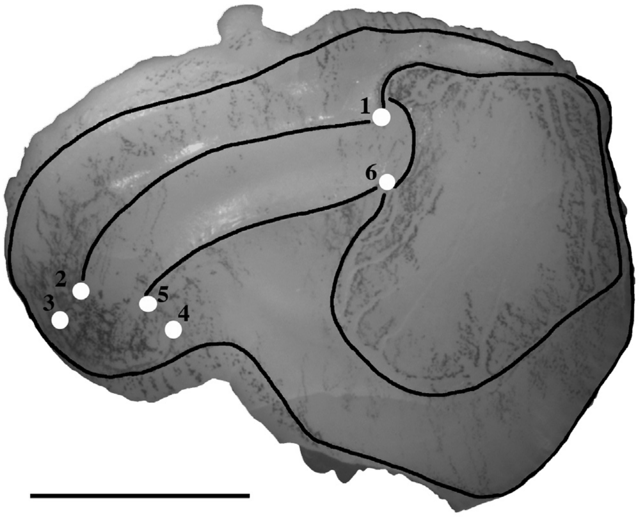

Figure 3. - Mesial view of the left sagitta showing the measurements taken on each otolith. Numbers refer to landmarks at the ostial-caudal joint (points 1 and 6) and at the end of the cauda (points 2 to 5). The sliding semi-landmarks align along the blue lines as follows: *ostium* outline: 85 semi-landmarks; precaudal depression edge: 9; caudal curves between *ostium* and caudal tip: 35 and 25; inner face rim: 100. Scale bar: 5 mm.

Table I. - Data for otolith reference collection.

Taxon	Locality	Number of otoliths (female, male)	Length range (mm)	Weight range (g)
Micropogonias altipinnis	Ecuador	21 (11, 10)	192-506 SL 237-600 TL	124-3800
	Peru	19		
Micropogonias manni	Chile	46 (24, 22)	214-451 SL 259-536 TL	192-1000

Sagittae data acquisition and analyses

I) We defined the position of landmarks and sliding semi-landmarks on the sagitta mesial surface using the criterion of homology. II) Left sagittae were stained with pencil graphite, oriented with their mesial surface up (Fig. 3), and observed under a binocular macroscope Leica Z6 coupled with a camera Leica DFC420 of 5 MP resolution. To evaluate the observer effect, we selected three sagittae of similar size and conservation state, and each was photographed 10 times. III) We placed the landmarks and the sliding semi-landmarks manually on each photo using Tps Dig2 v. 2.05 (Rohlf, 2005) as follows: two landmarks at the ostial-caudal joint and four landmarks at the end of the cauda (Fig. 3). The sliding semi-landmarks were placed on the *ostium* outline (85), at the precaudal depression edge (9), at the caudal curves between the *ostium* and the "buffer" shape caudal tip (35 + 25), and finally at the inner face rim (100). At this moment of the procedure the total number of points was fixed to 255 (six landmarks and 249 sliding semi-landmarks) using the criterion of the minimum number of points needed to properly define or describe sagitta inner shape; IV) Landmark and semi-landmark positions were translated, scaled (to unit centroid size) and rotated through generalized procrustes analysis (GPA, Rohlf and Slice, 1990) performed with Tps Relw v.1.36 program (Rohlf, 2004). Sliding semi-landmarks superposition includes the "slide" phase. It allows a superimposition of landmarks and sliding semi-landmarks as equivalents (Cucchi *et al.*, 2011; Gunz and Mitteroecker, 2013). Once landmark and semi-landmark positions are optimally adjusted, minimizing the bending energy between the consensus and each specimen (Gunz and Mitteroecker, 2013), giving a homolog geometric position of the entire set of points, they can be statistically analysed in a classical geometric morphometric framework (Adams *et al.*, 2004; Zelditch, 2012). We incorporated the final shape coordinates of the configuration into subsequent analyses as well as centroid size for each specimen. To evaluate the observer effect we performed a Principal Components Analysis (PCA) to quantify and visualize shape differences between the specimens. The PCA (data not shown) showed that shape variability due to repetitions was lower than shape variability between specimens, hence the measures are reliable.

Shape statistical analyses were performed with R v3.0.2 (R Core Team, 2013) using "Rmorph" library (Baylac, 2012). Because sliding semi-landmarks generate many variables, we used dimensionality reduction method (Baylac and Friess, 2005) that consists in a substitution of the number of predictors used in a Linear Discriminant Analysis (LDA) by the scores of the first axes of the PCA. We then worked on the first 16 axes (that represent 90% of shape variability) to achieve a correct balance to the number of sagittae (Evin *et al.*, 2013). First, we tested significant differences in shape and size between species and sex using MANOVA and ANOVA, respectively, with shape and centroid size as the dependent variable and the species as a factor. We also visualized shape differences by the deformations along the factorial axes calculated by multivariate regressions (Monteiro, 1999). We performed a LDA on shape (Evin *et al.*, 2013) to minimize intra-group variance and maximize the species separation, but also to visualize the morphological average deformations related to the discriminant axis. We calculated and analysed the discrimination level (percentage of individuals correctly classified by species).

Lastly, we analysed and visualized the relation between otolith shape and specimen external morphology (TL, SL and BW) using multivariate regressions (Monteiro, 1999) to complement our sagitta shape study.

RESULTS

Sagitta shape description

M. manni sagitta is more compressed in the front and stretched in the back than *M. altipinnis* sagitta (Fig. 4). The dorsal line separating the smooth inner face from the more granulate dorsal margin is more curved toward the center of the sagitta in *M. manni*. The *sulcus acusticus* also differs between the two species. The *ostium* is relatively more auriculated dorsally in *M. manni* and the ventral notch is relatively more pronounced. The precaudal depression edge is relatively more concave in *M. manni* and the caudal tip is more extended.

Results of the tests and discrimination rate

Otolith centroid size is not significantly different between the two species and between sexes (species: ANOVA $p = 0.132$; sex: ANOVA $p = 0.466$). Shape statistically differs between species (MANOVA $p < 0.001$) but not between sex (MANOVA, $p = 0.315$). Within each species, we also found that sex has no significant effect on shape of the sagitta mesial face (*M. altipinnis,* MANOVA, $p = 0.505$; *M. manni,* $p = 0.3$) nor on size (*M. altipinnis,* ANOVA, $p = 0.593$; *M. manni,* $p = 0.139$).

Regarding assignation results, we obtained high discrimination rate of sagitta shape, even though one *M. altipinnis* sagitta and two *M. manni* sagittae were not correctly assigned (Tab. II; Fig. 4A).

Sagitta shape and body variables

We found a significant correlation between sagitta shape and specimen body variables (TL, SL, WB), as clearly visualized by the projections (Tab. III; Fig. 5). The TL explained the larger part of shape variations, while most differences observed in relation to body weight could arise from the large interval of weight values from the specimens. However, all differences observed in relation to TL and SL indicate that otolith growth is localized mostly in the posterior and dorsal margins (Fig. 5), and the general tendency observed corresponds to a relative increase of the ostium and a localized growth of the dorsal margin which decrease the relative width of the otolith from larger and heavier specimens.

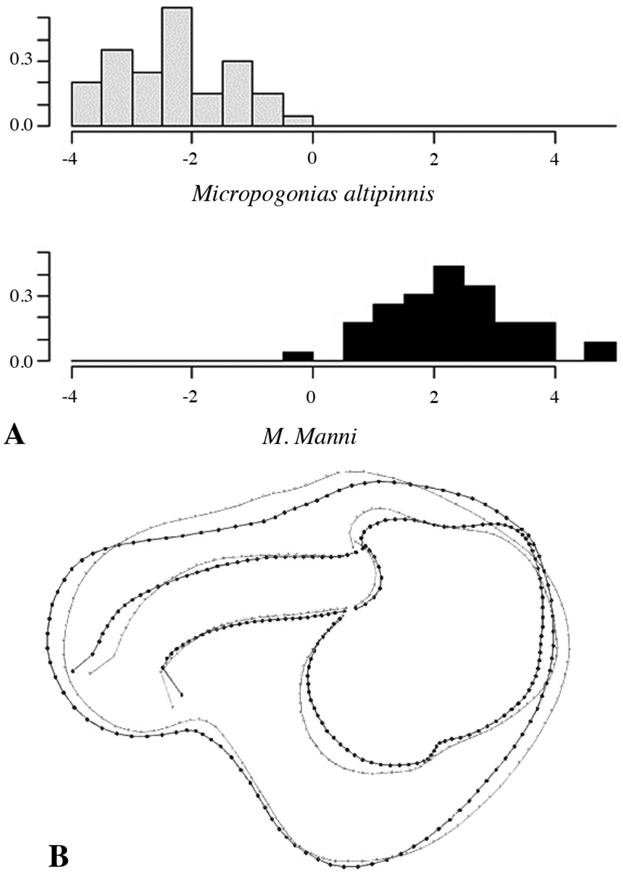

Figure 4. - Discriminant shape analysis between *Micropogonias altipinnis* and *M. manni* sagittae. **A**: Histogram showing the values of the intra- and inter-specific morphological variability. The x-axis shows the discriminant axis and the y-axis the frequency of distribution of sagittae of *M. altipinnis* and *M. manni*. **B**: Mean sagittae shape of *M. altipinnis* (in gray) and *M. manni* (in black).

Table II. - Number of sagittae with correct specific assignation (%).

Taxon	Number of sagittae correctly assigned/number studied	Shape discrimination percentage
Micropogonias altipinnis	39 / 40	97.5%
Micropogonias manni	44 / 46	95.6%
Total	83 / 86	96.5%

Table III. - *Micropogonias* sagitta shape and relationships of specimen body variables: weight (cubic); standard length (logarithm) (SL); total length (logarithm) (TL). P-value is the R^2 associated probability.

Relation between body variables and sagitta shape	P-value	R^2
BW and shape	< 0.001	$R^2 = 0.5597$
Log SL and shape	< 0.001	$R^2 = 0.5985$
Log TL and shape	< 0.001	$R^2 = 0.5856$

DISCUSSION

This study revealed (1) clear shape differences between sagittae of modern *Micropogonias* species and (2) sagitta shape variations related with morphology of specimens. These differences confirm what is known from the literature that recognizes sagitta as an important tool for sciaenid systematics (Sasaki, 1989; Schwarzhans, 1993). Similar observations were obtained by Ponton (2006) for Engraulidae and by Wakefield *et al.* (2014) for Etelinae.

Popper and Lu (2000) propose that interspecific differences in sagitta shape and size have a functional significance bearing on what and how fishes hear. Lombarte and Lleonart (1993) suggest that sagitta shape is regulated by genetics while size is influenced by environmental conditions. Interspecific differences observed in sagitta shape are localized on the margins and also in the anterior (*ostium*) and posterior (*cauda*) portions of the *sulcus acusticus* (Fig. 4B). This part of the sagitta is in contact with the macula through cilia. This connection is related to the hearing of the fish, which in turn depends on the environment under which the species develops (Popper and Lu, 2000; Popper *et al.*, 2005; Ramcharitar *et al.*, 2006). In fact, the two studied species inhabit different environments, one in the sea and one in coastal lakes, and likely have distinct feeding behaviours.

Lombarte and Lleonart (1993) argue that the sulcus morphology reflects phylogenetics relationships, that we could use to interpret the high percentage of sagittae correctly assigned to the species evaluated. The entire sagitta mesial surface is involved in the interspecific differences observed and could be used to accurately describe each species. The graphical representation of shape results is an important visual aid as shape is captured in a great detail through combined landmarks and sliding semi-landmarks shape analyses. As proposed by Gunz *et al.* (2012), sliding semi-landmarks constitute a very useful tool in taxonomic studies of specimens. Our results confirm that otolith shape could be used to obtain complementary characters that would reveal differences between *M. manni* and *M. furnieri*.

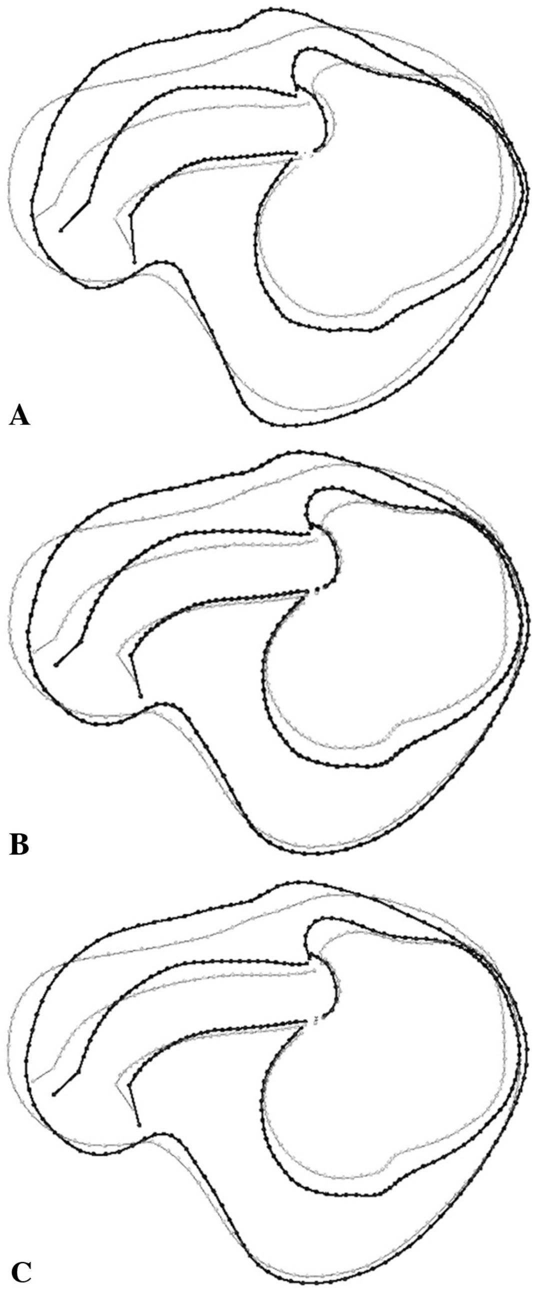

Figure 5. - Otolith shape variation associated with **A**: body-weight; **B**: standard length; **C**: total length of specimens. Grey lines correspond to low BW and SL/TL; black lines correspond to high BW and SL/TL.

Sagitta variations linked with sex were tested here. *Micropogonias* sex had no significant effect on shape or on centroid size, in contrast with Galley *et al.* (2006), finding that for *Gadus morhua* Linnaeus, 1758 differences in otoliths shape between specimens could be attributed to growth rate differences between males and females rather than to the stock origin. The only sexual differences remarked within sciaenid members concern the drumming muscles (Chao, 1995), which are always present in males but occasional in females (Ramcharitar *et al.*, 2006).

We showed here a significant correlation between the sagitta shape and the three body variables (Fig. 5). Monteiro *et al*. (2005) suggested that these ontogenetic variations might generate functional differences of hearing of fishes of different sizes, which could have an importance in localizing the sound source, but this needs to be clarified by further research (Monteiro *et al*., 2005).

To conclude, we showed that our protocol was adequate to distinguish the two species and also to quantify and visualize the relationship between sagitta shape and size of specimens. We also propose a reliable model of comparison especially useful when otoliths are isolated from the rest of the specimen. Archaeological remains will be an excellent subject for application of our method (Evin *et al*., 2013; Ros *et al*., 2014; Cornette *et al*., 2015). Since *Micropogonias* remains are common in coastal archaeological sites of the neotropics (Béarez *et al*., 2011; Goepfert *et al*., 2014), an accurate modern sagitta shape model will help to (1) attribute the archaeological pieces to a modern, known and correctly identified species and also (2) use shape differences to study paleoenvironmental conditions (Scartascini *et al*., 2009). Such fine identifications will allow more precise interpretation of the human prehistoric life mode, especially concerning fishing, but also of the paleobiodiversity (Llagostera, 1979; Chauchat *et al*., 2004; Béarez *et al*., 2011).

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REFERENCES

ADAMS D.C., ROHLF F.J. & SLICE D.E., 2004. - Geometric morphometrics: ten years of progress following the “revolution”. *Ital. J. Zool*., 71: 5-16.

BAYLAC M. & FRIESS M., 2005. - Fourier descriptors, Procrustes superimposition, and data dimensionality: an example of cranial shape analysis in modern human population. *In:* Modern Morphometrics in Physical Anthropology (D.E. Slice, ed.), pp. 145-165. New York: Kluwer Academic / Plenum Publishers.

BAYLAC M., 2012. - Rmorph: a R geometric and multivariate morphometrics library. Available from the author at baylac@mnhn.fr.

BÉAREZ P. & SCHWARZHANS W., 2013. - *Robaloscion*, a new genus for *Sciaena wieneri* Sauvage, 1883 (Teleostei, Sciaenidae) from the southeastern Pacific, with clarification of the taxonomic status of *Sciaena starksi* Evermann & Radcliffe, 1917. *Cybium,* 37(4): 273-279.

BÉAREZ P., DUFOUR E., CRÉDOU J. & CHAUCHAT C., 2011. - Les Paijaniens de la pampa de Los Fosiles (nord du Pérou, 11000-8000 BP) : pêcheurs, chasseurs ou opportunistes? *In*: Peuplements et préhistoire en Amériques (Vialou D., dir.), pp. 233-246. Paris: Éditions du CTHS.

BOOKSTEIN F., 1991. - Morphometric tools for landmark data: geometry and biology. 415 p. New York: Cambridge Univ. Press.

BROWNE P., JEFFREY L.L. & ROBERT L.D., 2002. - Improving pinniped diet analyses through identification of multiple skeletal structures in fecal samples. *Fish. Bull*., 100: 423-433.

CAMPANA S. & CASSELMAN J., 1993. - Stock discrimination using otolith shape analysis. *Can. J. Fish. Aquat. Sci.,* 50(5): 1062-1083.

CAÑÁS L., STRANSKY C., SCHLICKEISEN J., SAMPEDRO M.P. & FARIÑA C.A., 2012. - Use of the otolith shape analysis in stock identification of anglerfish (*Lophius piscatorius*) in the Northeast Atlantic. *J. Mar. Sci*., 69(2): 250-256.

CHAO L.N., 1978. - A basis for classifying Western Atlantic Sciaenidae (Teleostei: Perciformes). *NOAA Tech. Rep. NMFS Circ*., 415: 1-64.

CHAO L.N., 1986. - A synopsis on zoogeography of the Sciaenidae. *In:* Indo-Pacific Fish Biology (Uyeno T., Arai R., Taniuchi T. & Matsuura K., eds), pp. 570-586. Tokyo: Ichthyological Society of Japan.

CHAO L.N., 1995. - Sciaenidae. *In:* Guía FAO para la Identificación de Especies para los Fines de la Pesca, Pacifico Centro-Oriental, Part 2: Vertebrados (Fischer W., Krupp F., Schneider W., Sommer C., Carpenter K. & Niem V., eds), pp. 1427-1518. Rome: FAO.

CHAUCHAT C., PELEGRIN P., GALVEZ-MORA C., BECERRA-URTEAGA R. & ESQUERRE-ALVA R., 2004. - Projectile Point Technology and Economy. A Case Study from Paiján, North Coastal Peru. 126 p. College Station: Texas A&M Univ. Press.

CORNETTE R., HERREL A., STOETZEL E., MOULIN S., HUTTERER R., DENYS C. & BAYLAC M., 2015. - Specific information levels in relation to fragmentation patterns of shrew mandibles: do fragments tell the same story? *J. Archaeol. Sci*., 53: 323-330.

CUCCHI T., HULME-BEAMAN A., YUAN J. & DOBNEY K., 2011. - Early Neolithic pig domestication at Jiahu, Henan Province, China: clues from molar shape analyses using geometric morphometric approaches. *J. Archaeol. Sci*., 38: 11-22.

DERBY C.E. & LOVVORN J.R., 1997. - Comparison of pellets versus collected birds for sampling diets of double-crested cormorants. *Condor*, 99: 549-553.

ESCHMEYER W.N. & FONG J.D., 2015. - A Guide to Fish Collections in the Catalog of Fishes. Online version, updated 2 September 2015. Internet publication, San Francisco (California Academy of Sciences).

http://research.calacademy.org/research/ichthyology/catalog/collections.asp.

EVIN A., CUCCHI T., CARDINI A., STRAND VIDARSDOTTIR U., LARSON G. & DOBNEY K., 2013. - The long and winding road: identifying pig domestication through molar size and shape. *J. Archaeol. Sci*., 40(1): 1-9.

GALLEY E.A., WRIGHT P.J. & GIBB F.M., 2006. - Combined methods of otolith shape analysis improve identification of spawning areas of Atlantic cod. *ICES J. Mar. Sci*., 63: 1710-1717.

GOEPFERT N., BÉAREZ P., CHRISTOL A., GUTIÉRREZ B. & WUSCHER P., 2014. - The fishermen of Sechura: excavations at a specialised site from the Early Intermediate Period, extreme northern Peru. Antiquity Project Gallery, 340. Available at: http://journal.antiquity.ac.uk/projgall/goepfert340.

GUNZ P. & MITTEROECKER P., 2013. - Semilandmarks: a method for quantifying curves and surfaces. *Hystrix*, 24(1): 103-109.

GUNZ P., RAMSIER M., KUHRIG M., HUBLIN J.J. & SPOOR F., 2012. - The mammalian bony labyrinth reconsidered, introducing a comprehensive geometric morphometric approach. *J. Anat*., 220(6): 529-543.

LLAGOSTERA A., 1979. - 9,700 years of maritime subsistence on the Pacific: an analysis by means of bioindicators in the North of Chile. *Am. Antiquity,* 44(2): 309-324.

LLAGOSTERA A., KONG I. & IRATCHET P., 1997. - Análisis ictioarqueológico del sitio La Chimba 13 (II Región, Chile). *Chungara*, 29(2): 163-179.

LOMBARTE A. & LLEONART J., 1993. - Otolith size changes related with body growth, habitat depth and temperature. *Environ. Biol. Fish.*, 37: 297-306.

LOMBARTE A., CHIC Ò., PARISI V., OLIVELLA R., PIERA J. & GARCÍA E., 2006. - A web-based environment for shape analysis of fish otoliths. The AFORO database. *Sci. Mar.,* 70: 147-152.

MONTEIRO L., 1999. - Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Syst. Biol.*, 48(1): 192-199.

MONTEIRO L., GUILLERMO L., RIVERA L. & DI BENEDITTO A., 2004. - Geometric methods combining contour and landmark information in the statistical analysis of biological shape. *In:* Proceedings of the third Brazilian Symposium on Mathematical and Computational Biology (Mondaini R., ed.), pp. 336-355. Rio de Janeiro: E-papers.

MONTEIRO L., DI BENEDITTO A., GUILLERMO L. & RIVERA L., 2005. - Allometric changes and shape differentiation of sagitta otoliths in sciaenid fishes. *Fish. Res.,* 74: 288-299.

MORAIS P., RUFINO M.M., REIS J., DIAS E. & SOUSA R., 2013. - Assessing the morphological variability of *Unio delphinus* Spengler, 1783 (Bivalvia: Unionidae) using geometric morphometry. *J. Mollus. Stud.*, 80: 1-7.

MORENO C., 1970. - *Micropogon manni* n. sp. (Sciaenidae, Teleostomi). *Not. Mens. Mus. Nac. Hist. Nat.*, 170: 5-11.

MOTTA C.M., BICE A., BALASSONE G., BALSAMO G., FASCIO U., SIMONIELLO P., TAMMARO S. & MARMO F., 2009. - Morphological and biochemical analyses of otoliths of the ice-fish *Chionodraco hamatus* confirm a common origin with red-blooded species. *J. Anat.*, 214: 153-162.

NELSON J., 2006. - Fishes of the World. 4th edit., 622 p. Hoboken: John Wiley & Sons.

PONTON D., 2006. - Is geometrics efficient for comparing otolith shape of different fish species? *J. Morphol.*, 267: 750-757.

POPPER A. & LU Z., 2000. - Structure-function relationships in fish otolith organs. *Fish. Res.*, 46: 15-25.

POPPER A.N., RAMCHARITAR J. & CAMPANA S.E., 2005. - Why otoliths? Insights from inner ear physiology and fisheries biology. *Mar. Freshw. Res.*, 56: 497-504.

RAMCHARITAR J., GANNON D.P. & POPPER A.N., 2006. - Bioacoustics of Fishes of the Family Sciaenidae (Croakers and Drums). *Trans. Am. Fish. Soc.*, 135: 1409-1431.

R CORE TEAM, 2013. - A language and environment for statistical computing. R Foundation for Statistical Computing. Available at: www.R-project.org.

ROHLF F., 2004. - tpsRelw, relative warps analysis, version 1.36. Department of Ecology and Evolution, State Univ. of New York at Stony Brook.

ROHLF F.J., 2005. - tpsDig, digitize landmarks and outlines, version 2.05. Department of Ecology and Evolution, State Univ. of New York at Stony Brook.

ROHLF F.J. & SLICE D., 1990. - Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.*, 39: 40-59.

ROS J., EVIN A., BOUBY L. & RUAS M., 2014. - Geometric morphometric analysis of grain shape and the identification of two-rowed barley (*Hordeum vulgare* subsp. *distichum* L.) in southern France. *J. Archaeol., Sci.*, 41: 568-575.

SASAKI K., 1989. - Phylogeny of the family Sciaenidae, with notes on its zoogeography (Teleostei, Perciformes). *Mem. Fac. Fish., Hokkaido Univ.*, 36(1): 1-137.

SCARTASCINI F., CHARO M. & VOLPEDO A., 2009. - Caracterización de las estrategias de obtención de recursos ícticos a partir del análisis de otolitos. El caso de la costa norte del golfo San Matías (Río Negro). *In*: Arqueología de Patagonia: una Mirada desde el último Confín (SalemmeM., Santiago F., Álvarez M., Piana E., Vázquez M. & Mansur E., eds), vol. II, pp. 845-852. Ushuaia: Utopías.

SCHWARZHANS W., 1993. - A comparative morphological treatise of recent and fossil otoliths of the family Sciaenidae (Perciformes). Piscium Catalogus, Otolithi Piscium, 1: 1-245. Munich: Verlag F. Pfeil.

STEVENSON D.K. & CAMPANA S.E., 1992. - Otolith microstructure examination and analysis. *Can. Spec. Publ. Fish. Aquat. Sci.*, 117: 1-126.

TUSET V.M., LOZANO I.J., GONZALEZ J.A., PERTUSA J.F. & GARCIA-DIAZ M.M., 2003. - Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). *J. Appl. Ichthyol.*, 19: 88-93.

WAESSLE J.A., LASTA C.A. & FAVERO M., 2003. - Otolith morphology and body size relationships for juvenile Sciaenidae in the Río de la Plata estuary (35-36°S). *Sci. Mar.*, 67(2): 233-240.

WAKEFIELD C., WILLIAMS A., NEWMAN S., BUNEL M., DOWLING C., ARMSTRONG C. & LANGLOIS T., 2014. - Rapid and reliable multivariate discrimination for two cryptic Eteline snappers using otolith morphometry. *Fish. Res.*, 151: 100-106.

ZELDITCH M.L., SWIDERSKI D.L. & SHEETS H.D., 2012. - Geometric Morphometrics for Biologists: a Primer. 437 p. New York: Elsevier Academic Press.